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Kunzmann, A. T., McMenamin, U. C., Spence, A. D., Gray, R. T., Murray, L. J., Turkington, R. C., & Coleman, H. G. (2018). Blood Biomarkers for Early Detection of Oesophageal Cancer: A Systematic Review: A systematic review. *European Journal of Gastroenterology and Hepatology*, 30(3), 263-273.
<https://doi.org/10.1097/MEG.0000000000001029>

Published in:
European Journal of Gastroenterology and Hepatology

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

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BLOOD BIOMARKERS FOR EARLY DIAGNOSIS OF OESOPHAGEAL CANCER: A SYSTEMATIC REVIEW

Running head: Oesophageal cancer biomarkers review

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Disclosures: The project was kindly funded by **Ochre charity** (Registered charity number: SC032343).

The authors have no conflicts of interest to report.

Word count: 3445

ABSTRACT

Background: Oesophageal cancer prognosis remains poor due to the inability to detect the disease at an early stage. Non-tissue (serum, urinary or salivary) biomarkers potentially offer less invasive methods to aid early detection of oesophageal cancer. We aimed to systematically review studies assessing the relationship between non-tissue biomarkers and subsequent development of oesophageal cancer.

Methods: Using terms for biomarkers and oesophageal cancer, MEDLINE, EMBASE and WEB OF SCIENCE were systematically searched for longitudinal studies, published until April 2016, which assessed the association between non-tissue biomarkers and subsequent oesophageal cancer risk. Random effects meta-analyses were used to calculate pooled relative risk (RR) and 95% confidence intervals (CI), where possible.

Results: A total of 39 studies were included. Lower serum pepsinogen I concentrations were associated with an increased risk of oesophageal squamous cell carcinoma (n=3 studies, pooled RR=2.20, 95% CI: 1.31-3.70). However, the association for the pepsinogen I:II ratio was not statistically significant (n=3 studies, pooled RR=2.22, 95% CI: 0.77-6.40), with a large degree of heterogeneity observed ($I^2=68.0\%$). Higher serum glucose concentrations were associated with a modestly increased risk of total oesophageal cancer (n=3 studies, pooled RR=1.27, 95% CI: 1.02-1.57). No association was observed for total cholesterol and total oesophageal cancer risk (n=3 studies, pooled RR=0.95, 95% CI: 0.58-1.54). Too few studies assessed other biomarkers for meta-analyses.

Conclusions: Serum pepsinogen I concentrations could aid early detection of oesophageal squamous cell carcinoma. More prospective studies are needed to determine the use of other non-tissue biomarkers in the early detection of oesophageal cancer.

Keywords: serum; urinary; oesophageal adenocarcinoma; oesophageal squamous cell carcinoma

INTRODUCTION

Oesophageal cancer has a very poor prognosis, with less than 20% of patients surviving for more than 5 years from diagnosis [1,2]. Poor survival is attributable to late clinical presentation with advanced disease, with less than a quarter of cases diagnosed at stage I or II [3,4].

The two main histological subtypes, oesophageal adenocarcinoma (OAC) and oesophageal squamous cell carcinoma (OSCC), differ by aetiology and geographical incidence, with OAC more common in Western populations (>60% of oesophageal cancers) and OSCC more common (>90%) in Eastern populations, but share a poor prognosis [2,5,6].

Despite the high sensitivity of endoscopy as a diagnostic tool, a national screening programme for oesophageal cancer has not been justified due to the high cost, the invasiveness of the procedure and the psychological burden [7,8]. Cytosponge screening for Barrett's oesophagus and oesophageal cancer is currently being trialled in patients with heartburn symptoms [9,10], but otherwise the potential for viable screening options seems limited. Whilst endoscopy can also identify patients with Barrett's oesophagus (BO), the precursor lesion of OAC, or squamous dysplasia, the precursor lesion of OSCC, risk-stratification tools for identifying individuals at highest risk of neoplastic progression from limited. Surveillance programmes amongst BO patients place a considerable strain on gastroenterology resources and patients due to the limited risk-stratification[7].

Identification of cheaper, less invasive methods to identify individuals at risk of developing oesophageal cancer are needed to improve screening and surveillance options within the general public and patients with precancerous lesions, respectively [7]. Whilst tissue biomarkers offer promise in the early detection of oesophageal cancer, blood, urine or salivary tests could offer a simpler and less invasive alternative.

A large number of epidemiological studies have assessed the role of non-tissue biomarkers in oesophageal cancer risk [11,12]. However, many of the studies have been cross-sectional/retrospective, whereby the biomarker was used as an aid to oesophageal cancer diagnosis. Prospective studies are needed to determine if the changes in biomarkers occur early enough in cancer development to identify high risk individuals and aid early diagnosis.

An examination of biomarkers associated with oesophageal cancer may also provide insight into the mechanisms and aetiology of oesophageal cancer development.

The aim of this systematic review is to summarise evidence from all prospective studies which have assessed the relationship between blood, urinary and salivary biomarkers and risk of oesophageal cancer, in order to identify potential aides for risk stratification and early diagnosis of oesophageal cancer.

METHODS

LITERATURE SEARCH

To investigate blood, urine and salivary based biomarkers in the early diagnosis of oesophageal cancer, three databases, MEDLINE (1947-), EMBASE (1974-) and WEB OF SCIENCE (1980-), were systematically searched from inception to 29th April 2016 for primary studies in humans, published in any language. Databases were searched using exploded subject headings and combinations of keywords for biomarkers and oesophageal cancer (see Supplementary table 1 for full search terms). Reference lists and manual searches were used to identify any additional published studies.

ELIGIBILITY CRITERIA

Titles and abstracts of articles identified through the electronic search were reviewed independently, in duplicate, by two reviewers (from AK, HC, UM, RG, AS). The full text of articles for possible inclusion were then independently reviewed by two investigators (from AK, HC, UM, RG, AS). If there was disagreement between the reviewers at either stage, an independent review was undertaken by a third reviewer and a consensus reached.

Criteria for inclusion in the review included: assessment of a non-tissue (blood, urinary or salivary) based biomarker (including circulating epigenetic markers) in relation to total oesophageal cancer (any histological type), OAC or OSCC risk. Studies reporting only on oesophagogastric junction cancers or high grade dysplasia of the oesophagus, or not separating these from oesophageal tumours were not included. Included studies had to have at least a 6 month follow-up period after biomarker assessment

(to exclude prevalent cancers with diagnostic delays). Cross sectional studies where the biomarkers were measured at, or close to, the time of diagnostic assessment or following diagnosis were excluded. To ensure a narrow scope and to minimise repetition of existing systematic reviews,

oesophageal tissue biomarkers, blood-based genetic polymorphisms and infectious agents were not eligible for inclusion [13,14]. Studies using diagnoses of medical conditions in medical records as a proxy for biomarker status were not included due to potential differences in the exact biomarker measurement used for diagnosis (for example either high total cholesterol or high low-density lipoprotein (LDL) cholesterol could be used to diagnose hypercholesterolemia). Studies of biomarkers and oesophageal cancer mortality were not included. Participants included in individual studies could be healthy at biomarker assessment or have oesophageal conditions other than oesophageal cancer, such as Barrett's oesophagus.

DATA EXTRACTION

Data extraction was performed by an experienced author (AK). Extracted information from each of the individual studies included: author names and date of publication, study setting; study population, participant demographics and baseline characteristics; biomarkers assessed as well as the timing and method of assessment, the sample type; study methodology; recruitment and study completion rates and outcomes of interest. Articles in non-English languages were translated where necessary. The Newcastle-Ottawa scale for Cohort studies was used to assess study quality [15].

STATISTICAL ANALYSIS

Stata 14 software (Stata Corporation, College Station, TX, USA) was used for data analysis. Random effects meta-analyses were used to examine the pooled association between each biomarker and each type of oesophageal cancer (total, OAC or OSCC) if at least 3 eligible studies had assessed the association. Adjusted results were used where possible. A chi-squared test for heterogeneity was calculated and the I^2 statistic determined, to estimate the proportion of variation between study results attributable to heterogeneity rather than chance [16]. Heterogeneity was considered high if I^2 statistic was above 75% [16]. If studies only provided results stratified by participant characteristics (such as gender), the results were combined using preliminary meta-analysis prior to entry into the overall meta-analysis. Due to low study numbers identified for each biomarker,

publication bias could not be formally assessed [17]. Where studies included in meta-analyses used multiple classifications, or cut-offs for biomarkers concentrations (e.g. quartiles, categorical and continuous), we chose results based on similarity of classification to other studies (the majority of studies used tertiles/quartiles).

RESULTS

The searches identified a total of 15,334 study records (Medline=3210, Embase=6414, Web of Science=5710). After removal of duplicates, 6,995 studies remained. After a review of titles and abstracts, 82 articles were chosen for full text review, from which 36 studies were eligible for inclusion. An additional three studies eligible for inclusion were identified by manual searches and review of reference lists [18–20], meaning a total of 39 studies [18–56] were included in the review (Figure 1 and Table 1) and are further described by broad classification of biomarker type.

Fifteen studies were conducted in Eastern or South-East Asia populations, 12 studies were conducted in European populations and eight studies were conducted in North American populations. Seven studies were conducted amongst males only. The studies were mostly conducted within cohorts with a mean BMI below 25, though some studies were conducted amongst cohorts with Barrett's oesophagus at baseline and had average BMI above 25 (Table 1). Most studies were judged to be of a good (n=31 studies) or fair quality (n=6 studies), though two studies assessing exposure to toxic chemical within worker cohorts were judged to be poor quality (Table 2).

Lipid & glucose related biomarkers

We identified seven studies that assessed the association between serum lipid or glucose biomarkers and risk of oesophageal cancer (Supplementary table 2).

A meta-analysis of five studies [18,19,21–23] found no association between total serum cholesterol concentrations and risk of total oesophageal cancer (pooled RR=1.00, 95% CI: 0.79-1.27, $I^2=36.8\%$, Figure 2). Additionally, Lindkvist et al [54] provided a breakdown by type of oesophageal cancer and found no association between high total cholesterol and risk of either OAC or OSCC, (Supplementary table 2).

Wulaningsih et al [23] also tested subtypes of cholesterol related measures, though could not adjust for body mass index. Strong inverse associations with risk of oesophageal cancer were found for LDL

cholesterol, the LDL:HDL ratio and the Apo B:ApoA-1 ratio in that study. No associations were seen for HDL cholesterol, total:HDL cholesterol ratio, the triglyceride:HDL ratio, ApoB, or Apo A concentrations. A positive association was observed for triglyceride concentrations as individuals in the highest quartile of triglyceride concentrations had over double the risk of oesophageal cancer. However, Lindkvist et al [54] found a weaker association for triglycerides, which was not statistically significant for either OAC or OSCC. Ahn et al [21] found a non-significant positive association between HDL cholesterol concentrations and risk of oesophageal cancer.

Wulaningsih et al [23], Stocks et al[20] and Jee et al[52] all assessed the association between glucose concentrations and oesophageal cancer risk. A meta-analysis of these three studies found a 27% increase in risk of total oesophageal cancer in individuals with higher glucose concentrations (pooled RR=1.27, 95% CI: 1.02-1.57) with no evidence of heterogeneity ($I^2=0.0\%$) (Figure 3).

Only Lindkvist et al[54] provided a breakdown by cancer type with the results indicative that glucose concentrations were more strongly associated with OSCC risk than OAC risk.

Duggan et al[55] assessed the association between HOMA (Homeostatic model assessment) scores, calculated by multiplying insulin and glucose concentrations, and risk of oesophageal adenocarcinoma amongst a cohort of individuals with Barrett's oesophagus and found evidence of an association when assessed continuously (RR=1.64, 95% CI: 1.08-2.48).

Nutrition-related biomarkers

We identified 16 studies that assessed the association between nutrition-related biomarkers and oesophageal cancer (Supplementary table 3).

Eight studies assessed the role of nutrition-related biomarkers and total oesophageal cancer risk. Kimm et al [35] found that individuals with higher aspartate transaminase: alanine transaminase (AST:ALT) ratios (a biomarker associated with liver damage often caused by alcohol consumption)

had a higher risk of oesophageal cancer. Tsuboya et al [46] found no association between gamma-glutamyltransferase concentrations (associated with excessive alcohol intake and liver disease) and risk of total oesophageal cancer. Knekt et al [56] did not find an association for beta-carotene and the others have not been investigated previously. No associations were observed for alpha-tocopherol [56], selenium [56], retinol [56], tea polyphenols [36], calcium [33] and phosphate [34] and risk of total oesophageal cancer.

Eight studies assessed the role of nutrition-related biomarkers in OSCC risk and three studies assessed the role of nutrition-related biomarkers in OAC risk. Murphy et al [43] found a significant reduction in OSCC risk amongst individuals with higher serum cysteine concentrations. Fanidi et al [42] found a significant increase in OSCC risk amongst individuals with higher serum folate concentrations but not other b-vitamins, or one-carbon products (homocysteine/methionine). Some of the results from studies assessing OSCC risk contrast with the results from separate studies of total oesophageal cancer described earlier, with significant inverse associations seen for selenium [41] and alpha-tocopherol [38], but null results for beta-carotene and beta-cryptoxanthin [39]. Null associations were also seen in individual studies for vitamin C, retinol and lutein [39,40,56]. Chen et al [53] found evidence of an association between serum 25(OH)D and OSCC, however, Abnet et al [32] did not find an association with OSCC or OAC in an analysis of data from 8 prospective cohorts. Takata et al [37] also found no association between serum selenium concentrations and risk of progression to OAC amongst a cohort of individuals with Barrett's oesophagus.

Metabolic/digestive related biomarkers

We identified nine studies identified that assessed the association between biomarkers related to metabolism or inflammation and oesophageal cancer risk (Supplementary table 4).

A random effects meta-analyses of 3 studies [27–29] found that lower pepsinogen I (PGI) concentrations were associated with increased risk of OSCC (pooled RR=2.20, 95% CI: 1.31-3.70), with little heterogeneity observed ($I^2=9.3\%$) (Figure 4).

The same studies [27–29] also assessed the association between the PGI: PGII ratio and risk of OSCC but did not find evidence of an association (pooled RR=1.44, 95% CI: 0.42-4.91), with a large degree of heterogeneity ($I^2=75.7\%$) (Figure 5). Sensitivity analyses indicated that heterogeneity was lowered after the exclusion of results from Cook et al [27] (pooled RR=0.91, 95%CI: 0.37-2.20, $I^2=18.3\%$).

Duggan et al [55] found a significant positive linear association between serum leptin concentrations, but not adiponectin, and risk of OAC in individuals with Barrett's oesophagus, including when limiting to cancers diagnosed within 3 years of baseline blood sampling (HR=2.51, 95% CI: 1.09-5.81). However, the association was only statistically significant in continuous analysis and has yet to be tested in other studies. No studies identified assessed leptin and adiponectin and OSCC risk.

De Martel et al [24] found a borderline association between high serum ghrelin concentrations and lower OAC risk in Barrett's oesophagus patients. Similarly, Murphy et al [30] found lower ghrelin levels were associated with an increased risk of OSCC.

Inflammation-related biomarkers

Keeley et al [31] found evidence of strong positive associations between various inflammation-related biomarkers, including Tumour Necrosis Factor (TNF)-alpha, TNF-beta, MCP-3 (Monocyte chemotactic protein), IL-1R, Interleukin (IL)-13 and GM-CSF (granulocyte/macrophage colony-stimulating factor) , and OSCC risk.

Two studies assessed the association between inflammation-related biomarkers in Barrett's oesophagus cohorts. Hardikar et al [25] found no evidence of an association between C-Reactive Protein, IL6, Isoprostane, TNF-alpha or TNF-beta and risk of progression to OAC. Siahpash et al [26] found no evidence of an association between insulin-like growth factor 1 and risk of OAC.

Miscellaneous biomarkers

Results of individual studies examining the association between miscellaneous biomarkers and risk of oesophageal cancer are listed in Supplementary table 5. Haemoglobin was not associated with risk of oesophageal cancer, even when limiting the time from measurement to diagnosis to 3 years, although there was evidence of a trend which suggested a stronger association when limiting to oesophageal cancer diagnoses within a year of biomarker measurement [45].

Leukocyte telomere length was found to have a strong association with risk of progression to oesophageal adenocarcinoma amongst a cohort of individuals with Barrett's oesophagus in one study [49] but requires confirmation in other studies. However, lymphocyte mutagen sensitivity, a marker for the ability to repair DNA damage, was not found to be associated with risk of progression amongst the same Barrett's oesophagus cohort [50].

Biomarkers of smoking behaviours such as total N'-nitrosonornicotine (NNN), a tobacco-specific carcinogen, were found to be associated with risk of oesophageal cancer amongst male smokers in one study [44].

No association was found between sphingosines and risk of OSCC [51].

Exposure to toxins was also investigated, though neither urine levels of lead or trichloroethylene amongst workers in jobs at risk of exposure to these chemicals were found to be associated with oesophageal cancer risk [47,48].

DISCUSSION

This systematic review sought to examine non-tissue biomarkers associated with oesophageal cancer risk, in order to identify biomarkers that could be used to predict oesophageal cancer development. The review identified 39 studies which assessed the role of various biomarkers in oesophageal cancer risk. Meta-analyses revealed an association between serum PGI concentrations and OSCC risk (n=3 studies) and between serum glucose concentrations and total oesophageal cancer risk (n=3 studies). No associations were observed for total cholesterol and total oesophageal cancer risk (n=5 studies). However, there were too few studies to conduct meta-analyses for the majority of biomarkers and biomarkers with promising cross-sectional findings remain to be tested in prospective analyses.

The association between PGI concentrations was consistent across the three studies [27–29] included in the meta-analysis and indicated that individuals with lower PGI concentrations had over double the subsequent risk of OSCC. The association was similar between two studies in China and one in Finland (limited to males) suggesting geographical variation was not indicated. Similar effect sizes were observed in a meta-analysis of the same three studies assessing the association between the PGI:PGII ratio and OSCC risk, though the result was not statistically significant and heterogeneity was high. The results are supported by a study indicating an association between pepsinogen concentrations and risk of esophageal squamous dysplasia [57]. Lower PGI concentrations and a lower PGI:PGII ratio are thought to be indicators of gastric atrophy [58,59]. Lower PGI concentrations could be related to OSCC through bacterial overgrowth resulting from the reduced gastric acid production during gastric atrophy [60–62] or duodenal reflux [27,63]. Gastric atrophy is often caused by *Helicobacter pylori* (*H. pylori*) infections, and *H. pylori* infection has previously been linked to OSCC risk in Eastern populations [64]. However, the association between PGI and OSCC risk appears to be independent of *H. pylori* status as the studies included in the current review either adjusted for *H. Pylori* or did not find similar evidence of an association between *H. pylori* and OSCC

risk in separate analyses. Though serologic amnesia is possible, the results suggest that pepsinogen measurement could potentially aid OSCC risk-stratification independently of *H. pylori* measurement by identifying individuals at higher OSCC risk. PGI and the PGI:PGII ratio have previously been considered as part of gastric cancer screening programs in some countries [58,59,65]. However, questions remained about the cost-effectiveness. Should later studies confirm the results of the current meta-analysis, policy makers could assess the potential additional benefit of early OSCC detection when assessing whether to implement cancer screening programmes measuring pepsinogen concentrations/ratios, particularly in high-risk areas for OSCC [65].

The lack of an association between total cholesterol concentrations and risk of total oesophageal cancer in a meta-analysis of five studies, could be due to the different actions of each type of cholesterol. Wulaningsih et al [23] found stronger inverse association between more specific cholesterol markers, including LDL cholesterol, the ratio of LDL:HDL cholesterol and the ApoB:ApoA ratio, and oesophageal cancer risk but were unable to adjust for BMI which could confound the associations. Therefore, whilst an association between total cholesterol concentrations and oesophageal cancer risk appears unlikely, further detailed investigations into the association between more specific lipid biomarkers and oesophageal cancer risk independently of BMI seem warranted.

The association between serum glucose and total oesophageal cancer risk was modest (27%), but notably consistent across the three studies in the meta-analysis, despite geographical differences (Two studies from Europe and one study from South Korea). Two of the 3 studies included adjustment for BMI [54] or reported no change after adjustment for BMI [52], suggesting the results may be independent of BMI and could add predictive value to potential oesophageal cancer risk stratification models. The findings are consistent with the results of a systematic review which found a 27% increased risk of oesophageal cancer in individuals with diabetes mellitus in a pooled analysis of cohort studies [66]. However, the similarity could arise as the studies included in the current

review used measurements recorded in clinical databases so individuals may have been deemed to be at high risk of diabetes, meaning the results may not be representative of low-risk individuals. The result is also consistent with an observation by Duggan et al [55] of an association between HOMA scores, calculated by multiplying serum insulin and glucose concentrations, and risk of oesophageal adenocarcinoma. The potential mechanism mediating the association between serum glucose concentrations and oesophageal cancer could be oxidative stress related to hyperglycaemia or growth promotion by insulin-like growth factor (IGF) [67]. However, for the latter, it is important to note that no association was observed between IGF and OAC risk in a study by Siahpush et al [26] included in the current review, despite promising findings from non-prospective studies [68]. Only one study in the current review provided a breakdown by histological subtype of oesophageal cancer, therefore more studies will be needed to assess whether the associations with serum glucose are similar for both OAC and OSSC. Nevertheless, the review suggests that serum glucose concentrations could potentially aid risk stratification of individuals for oesophageal cancer risk, especially given the simplicity of blood glucose testing. However, a check of diabetes history may suffice and the modest strength of the association with oesophageal cancer risk means that the potential clinical benefit in terms of identifying high-risk individuals is likely to be limited unless incorporated into a wider panel of biomarkers or risk-stratification measures.

Despite the large number of biomarkers assessed, the majority of biomarkers have been tested by too few studies for strong conclusions to be formed, perhaps due to the difficulty in assessing rarer cancers such as oesophageal cancer in prospective studies. None of the studies identified assessed salivary biomarkers and very few assessed urinary biomarkers, despite promising findings for other gastrointestinal cancers [69]. For other novel blood markers, such as serum anti-p53 and micro RNAs, recent evidence from cross-sectional studies suggests that serum concentrations differ between oesophageal cancer patients and cancer-free controls [12,70,71], but no prospective studies have been conducted so these biomarkers were not included in this review. Further studies

are needed to assess non-tissue biomarkers given the public and clinical demand for blood based biomarkers [72,73].

The review provides a comprehensive assessment of the existing evidence of non-tissue biomarkers in oesophageal cancer risk and has identified promising associations between PGI concentrations and OSCC risk. A limitation was that many studies examined total oesophageal cancer risk without providing separate results for OAC and OSCC, despite the substantial differences between OAC and OSCC [74] in terms of biology, risk factors and patterns of incidence, possibly in order to maximise statistical power. Many of the included studies aimed to identify aetiological rather than early diagnostic biomarkers, which precluded an examination of the association with oesophageal cancers occurring within a period close to biomarker measurement (<3 years) useful for early detection [45]. Similarly, the biomarkers assessed were only measured at one point in time, which could also limit the ability to identify the optimal time-point of biomarker measurement

In summary, some non-tissue biomarkers, such as serum PGI concentrations may offer promise in identifying individuals at risk of oesophageal cancer which could help target screening or surveillance programmes and aid in the early detection of oesophageal cancer. However, too few studies have assessed the same biomarkers, and some promising biomarkers remain to be tested prospectively in order to adequately develop a biomarker panel for risk-stratification or for use in future oesophageal cancer screening programmes.

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Tables:

Table 1. Characteristics of studies included in review

Author (year)	Cohort name	Country	Study	Type	Ag	Sex	BMI (average,	Follow-	Biomarkers measured	Bioma	Oesophageal cancer		
											Total	OAC	OSC
Abnet (2001)	General population trial	China	NCC	OSCC	40	All	n/s	5.25	Sphingolipids	Serum			Yes
Abnet (2003)	General population trial	China	NCC	OSCC	40	All	22.1 (2.5)	5.25	Carotenoids	Serum			Yes
Abnet (2010)	Vitamin D pooling project	Multiple	NCC	All	18	All	25.3 (n/s)	1.7-11.8†	25(OH)D	Serum	Yes	Yes	Yes
Ahn (2009)	ATBC	Finland	Cohor	All	50	Men	n/s	14.9	Cholesterol	Serum	Yes		
Chao (2006)	Seattle Barretts esophagus	USA	Cohor	OAC	18	All	n/s	6.4†	Mutagen sensitivity	Serum		Yes	
Chen (2007)	General population trial	China	NCC	OSCC	40	All	n/s	5.25	25(OH)D	Serum			Yes
Chyou (1992)	Honolulu heart program	USA	Cohor	All	28	All	n/s	22 max	Cholesterol	Serum	Yes		
Cook (2010)	ATBC	Finland	NCC	OSCC	50	Men	26.4 (3.9)	14.9	Pepsinogens	Serum			Yes
De Martel	Kaiser Permanente	USA	NCC	OAC	18	All	n/s	19.7	Ghrelin	Serum		Yes	
Duggan (2013)	Seattle Barretts esophagus	USA	Cohor	OAC	18	All	29.4 (4.3)	6.7 ‡	Leptin, Adiponectin &	Serum		Yes	
Edgren (2010)	Scandinavian blood donors	Scandinavia	NCC	All	18	All	n/s	5 max	Haemoglobin	Serum	Yes		
Fanidi (2015)	EPIC	European	NCC	All	25	All	n/s	6.4 ‡	One carbon metabolites	Plasm		Yes	Yes
Gwini (2012)	Lead workers	Australia	Cohor	All	18	Men	n/s	16.2	Lead	Blood	Yes		
Hansen (2013)	Trichloroethylene workers	Scandinavia	Cohor	All	18	All	n/s	45 max	Trichloroethylene	Urinar	Yes	Yes	
Hardikar (2014)	Seattle Barretts esophagus	USA	Cohor	OAC	18	All	n/s	6.14†	Cytokines	Serum		Yes	
Hecht (2013)	Shanghai mens cohort	China	NCC	All	45	Men	n/s	12 max	Smoking biomarkers	Urinar	Yes		
Jee (2005)	National Health Insurance	South	Cohor	All	30	All	23.2 (2.6)	10 max	Glucose	Serum	Yes		
Keeley (2014)	Golestan cohort study	Iran	NCC	OSCC	40	All	n/s	9 max	Cytokines	Serum			Yes
Kimm (2010)	National Health Insurance	South	Cohor	All	30	Men	23.2 (2.7)	14 ‡	Alcohol biomarkers	Serum	Yes		
Kitahara (2011)	National Health Insurance	South	Cohor	All	30	All	23.2 (2.6)	12.7	Cholesterol	Serum	Yes		
Knekt (1991)	Social Insurance Institution	Finland	NCC	All	15	All	n/s	9 max	Micronutrients	Serum	Yes		
Lam (2013)	General population trial	China	NCC	OSCC	40	All	n/s	5.25	Vitamin C	Plasm			Yes
Lindkvist	Me-Can	Multiple	Cohor	Both	18	All	25.3 (4.0)	12	Cholesterol, glucose	Serum		Yes	Yes
Mark (2000)	General population trial	China	NCC	OSCC	40	All	n/s	5.25	Selenium	Serum			Yes
Murphy (2011)	General population trial	China	NCC	OSCC	40	All	21.8 (2.6)	5.25	Cysteine	Serum			Yes
Murphy (2012)	ATBC	Finland	NCC	OSCC	50	Men	26.6 (4.0)	14.9	Ghrelin	Serum			Yes
Ren (2009)	General population trial	China	Cohor	OSCC	40	All	22 (n/s)	5.25	Pepsinogens	Serum			Yes
Risques (2007)	Seattle Barretts esophagus	USA	Cohor	OAC	18	All	n/s	5.8	Leukocyte telomere length	Serum		Yes	
Siahpush	Seattle Barretts esophagus	USA	Cohor	OAC	18	All	28.7 (n/s)	5.8	Insulin-like Growth Factor	Serum		Yes	
Sun (2002)	Shanghai Mens cohort	China	NCC	All	45	Men	n/s	5.05	Tea polyphenols	Urinar	Yes		
Stocks (2009)	Me-Can	Multiple	Cohor	All	18	All	n/s	12.2	Glucose	Serum	Yes		
Strohmaier	Me-Can	Multiple	Cohor	All	18	All	25.3 (4.0)	11.7	Cholesterol	Serum	Yes		
Takata (2012)	Seattle Barretts esophagus	USA	Cohor	OAC	18	All	n/s	7.3 ‡	Selenium	Serum		Yes	
Taylor (2003)	General population trial	China	NCC	OSCC	40	All	n/s	5.25	Tocopherols	Serum			Yes
Tsuboya (2012)	Ohsaki cohort	Japan	Cohor	All	40	All	23.6 (3.0)	10 max	Gamma-	Serum	Yes		
Wulaningsih	AMORIS	Sweden	Cohor	All	20	All	n/s	12	Lipids & glucose	Serum	Yes		
Wulaningsih	AMORIS	Sweden	Cohor	All	20	All	n/s	12.75	Phosphate (inorganic)	Serum	Yes		
Wulaningsih	AMORIS	Sweden	Cohor	All	20	All	n/s	12.75	Calcium	Serum	Yes		
Xue (2013)	Hebei province	China	Cohor	OSCC	18	All	n/s	15 max	Pepsinogens	Serum			Yes

Studies were within cohorts not selected on basis of presence of precancerous condition (such as Barrett's oesophagus) unless indicated by †.

Study follow-ups reported as means unless stated otherwise, or medians if indicated by ‡

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; AMORIS: Swedish Apolipoprotein Mortality Risk study; ATBC: Alpha-tocopherol, Beta-caroten trial; EPIC: European Prospective investigation into Cancer and Nutrition; HOMA: Homeostatic model assessment; max: maximum; Me-Can: Metabolic Syndrome and Cancer cohort; NCC: Nested case-control; OAC: Oesophageal adenocarcinoma; OSCC: Oesophageal squamous cell carcinoma.

Table 2. Quality assessment using the Newcastle-Ottawa scale for cohort studies

Author (year)	Selection				Comparability	Outcome			Total	Grade ^c
	Representative	Selection of non-cases	Exposure ascertainment	Outcome not present	Adjustment/matching*	Outcome assessment	Follow-up length†	Loss to follow-up		
Abnet (2001)	0	1	1	0	1	1	1	1	6	Fair
Abnet (2003)	1	1	1	0	1	1	1	1	7	Good
Abnet (2010)	0	1	1	1	2	1	1	1	8	Good
Ahn (2009)	0	1	1	1	2	1	1	1	8	Good
Chao (2006)	1	1	1	1	2	1	1	1	8	Good
Chen (2007)	1	1	1	0	1	1	1	1	7	Good
Chyou (1992)	1	1	1	1	1	1	1	1	8	Good
Cook (2010)	0	1	1	1	2	1	1	1	8	Good
De Martel	0	1	1	1	2	1	1	1	9	Good
Duggan (2013)	1	1	1	1	2	1	1	1	9	Good
Edgren (2010)	1	1	1	1	1	1	1	1	8	Good
Fanidi (2015)	1	1	1	1	2	1	1	1	9	Good
Gwini (2012)	0	0	0	1	0	1	1	0	3	Poor
Hansen (2013)	0	0	1	1	0	1	1	1	5	Poor
Hardikar	1	1	1	1	2	1	1	1	9	Good
Hecht (2013)	0	1	1	1	2	1	1	1	8	Good
Jee (2005)	0	0	1	1	2	1	1	1	7	Fair
Keeley (2014)	1	1	1	1	2	1	0	1	8	Fair
Kimm (2010)	0	0	1	1	2	1	1	1	7	Fair
Kitahara (2011)	0	0	1	1	2	1	1	1	7	Fair
Knekt (1991)	1	1	1	1	1	1	1	1	8	Good
Lam (2013)	1	1	1	0	1	1	1	1	7	Good
Lindkvist	1	1	1	1	2	1	1	1	9	Good
Mark (2000)	1	1	1	0	1	1	1	1	7	Good
Murphy (2011)	1	1	1	0	1	1	1	1	7	Good
Murphy (2012)	0	1	1	1	2	1	1	1	8	Good
Ren (2009)	1	1	1	1	2	1	1	1	9	Good
Risques (2007)	1	1	1	1	2	1	1	1	9	Good
Siahpush	1	1	1	1	2	1	1	1	9	Good
Stocks (2009)	1	1	1	1	2	1	1	1	9	Good
Strohmaier	1	1	1	1	2	1	1	1	9	Good
Sun (2002)	1	1	1	1	2	1	1	1	9	Good
Takata (2012)	1	1	1	1	2	1	1	1	9	Good
Taylor (2003)	1	1	1	0	1	1	1	1	7	Good
Tsuboya (2012)	1	1	1	1	2	1	1	1	9	Good
Wulaningsih	0	1	1	1	1	1	1	1	7	Good
Wulaningsih	0	1	1	1	1	1	1	1	7	Good
Wulaningsih	0	1	1	1	1	1	1	1	7	Good
Xue (2013)	1	1	1	1	1	0	1	1	6	Fair

^a Age and sex=1, BMI/WHR for OAC/Smoking for OSCC=2^b 5 years+^c **Good quality:** 3 or 4 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain**Fair quality:** 2 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain**Poor quality:** 0 or 1 star in selection domain OR 0 stars in comparability domain OR 0 or 1 stars in outcome/exposure domain

FIGURE LEGENDS:

Figure 1. Numbers at each stage of the study selection process.

Figure 2. Forest plots from random effects meta-analysis for the association between total serum cholesterol concentrations and risk of oesophageal cancer (total). Columns indicate the number of cases and non-cases with low (reference category) and high total serum cholesterol, relative risk (95% confidence intervals) and weighting. Abbreviations: ES: Relative risk; CI: Confidence intervals.

Figure 3. Forest plots from random effects meta-analysis of the association between serum glucose concentrations and risk of oesophageal cancer (total). Columns indicate the number of cases and non-cases with low (reference category) and high total serum glucose concentrations, relative risk (95% confidence intervals) and weighting. Abbreviations: ES: Relative risk; CI: Confidence intervals.

Figure 4. Forest plots from random effects meta-analysis of the association between serum PGI concentrations and risk of oesophageal squamous cell carcinoma. Columns indicate the number of cases and non-cases with high (reference category) and low serum PGI concentrations, relative risk (95% confidence intervals) and weighting. Abbreviations: ES: Relative risk; CI: Confidence intervals; PG: pepsinogen.

Figure 5. Forest plots from random effects meta-analysis of the association between serum PGI:PGII ratio and risk of oesophageal squamous cell carcinoma. Columns indicate the number of cases and non-cases with high (reference category) and low serum PGI:PGII ratio , relative risk (95% confidence intervals) and weighting. Abbreviations: ES: Relative risk; CI: Confidence intervals; PG: pepsinogen.